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**A new class of temporarily phenotypic enhancers identified by CRISPR/Cas9-mediated genetic screening.**

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**Public Summary:**

With <2% of the human genome coding for proteins, a major challenge is to interpret the function of the noncoding DNA. Millions of regulatory sequences have been predicted in the human genome through analysis of DNA methylation, chromatin modification, hypersensitivity to nucleases, and transcription factor binding, but few have been shown to regulate transcription in their native contexts. We have developed a high-throughput CRISPR/Cas9-based genome-editing strategy and used it to interrogate 174 candidate regulatory sequences within the 1-Mbp POU5F1 locus in human embryonic stem cells (hESCs). We identified two classical regulatory elements, including a promoter and a proximal enhancer, that are essential for POU5F1 transcription in hESCs. Unexpectedly, we also discovered a new class of enhancers that contribute to POU5F1 transcription in an unusual way: Disruption of such sequences led to a temporary loss of POU5F1 transcription that is fully restored after a few rounds of cell division. These results demonstrate the utility of high-throughput screening for functional characterization of noncoding DNA and reveal a previously unrecognized layer of gene regulation in human cells.

**Scientific Abstract:**

With <2% of the human genome coding for proteins, a major challenge is to interpret the function of the noncoding DNA. Millions of regulatory sequences have been predicted in the human genome through analysis of DNA methylation, chromatin modification, hypersensitivity to nucleases, and transcription factor binding, but few have been shown to regulate transcription in their native contexts. We have developed a high-throughput CRISPR/Cas9-based genome-editing strategy and used it to interrogate 174 candidate regulatory sequences within the 1-Mbp POU5F1 locus in human embryonic stem cells (hESCs). We identified two classical regulatory elements, including a promoter and a proximal enhancer, that are essential for POU5F1 transcription in hESCs. Unexpectedly, we also discovered a new class of enhancers that contribute to POU5F1 transcription in an unusual way: Disruption of such sequences led to a temporary loss of POU5F1 transcription that is fully restored after a few rounds of cell division. These results demonstrate the utility of high-throughput screening for functional characterization of noncoding DNA and reveal a previously unrecognized layer of gene regulation in human cells.

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